Docket No.: D2033-701910/ 10280-053001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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| Applicant: | Robert C. Ladner et al. |
| Serial No: | 10/656,350 |
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| For: | DISPLAY LIBRARY PROCESS |
| Examiner: | Jeffrey S. Lundgren |
| Art Unit: | 1639 |

## DECLARATION OF ROBERT CHARLES LADNER, PH.D. UNDER 37 C.F.R. § 1.132

I, ROBERT CHARLES LADNER, pursuant to 37 C.F.R. § 1.132, declare the following:

1. My education and professional experience and qualifications are presented in the attached biographical sketch (Appendix). In addition, I am a named inventor on numerous patents in the phage display technology.

2. I am an employee of Dyax Corp., the assignee of this application.

3. I have reviewed the amended claims in this application and understand them to recite, *inter alia*:

- A method of selecting phage that encode a target binding protein from a plurality of display phage. The method includes the step of producing replicate phage from the infected cells in the presence of the target immobilized to the support, thereby forming replicate phage immobilized to the support via binding to the target, wherein the producing is completed in less than 4 hours;

- A method of identifying members of a bacteriophage library that have a desired binding property. The method includes the step of amplifying members of a subset of bacteriophage members in less than 4 hours; and

- A method of selecting a nucleic acid that encodes a binding protein from a library of display phage. The method includes the step of producing phage from the infected cells in the presence of the target, the produced phage being replicates of phage that bind to the target, wherein the producing is completed in less than 4 hours.

4. I have reviewed the Office Action mailed May 1, 2009 regarding the above-referenced application and understand that the Examiner has rejected the claims as allegedly obvious. The Office appears to rely on Janda (U.S. Pat. No. 5,571,681; hereinafter "the '681 patent") as supposedly rendering obvious the element of producing

or amplifying phage in less than 4 hours. In making this rejection, the Office also relies on Ladner (U.S. Pat. No. 5,403,484; hereinafter "the '484 patent") and Anderson (U.S. Pat. No. 6,649,419; hereinafter "the '419 patent").

For example, with respect to this element, the Office alleges at pages 11-12 of the Office Action:

> Although Ladner provides certain general guidelines and conditions for reaction times involving the phage, Ladner does not explicitly teach reaction times of less than 4 hours for step (e) or steps (d) and (f), as in claims 10 and 12, or the cycles being less than 8 hours as in claim 28; nor does Anderson. Ladner also does not explicitly teach a change in the temperature upon the producing step as in claim 14; nor does Anderson.
>
> Janda generally teaches the use of covalent conjugates that are immobilized by attachment to a substrate through a solid phase and are easily separated from unconjugated elements of the combinatorial library by stringent washing. Janda generally teaches combinatorial libraries employing phagemid-display are particularly preferred since such phagemids include genetic material for identifying and amplifying conjugated catalysts. In describing the reactions for contact phage with the host cell, incubating the cell, and expressing the phage in the host cell, the processes can be carried out in less than four hours, such as the 15 minutes to infect the XLI-Blue™ cells, and the 2 hour culturing - note that the overnight cell selection with kanamycin is not required due to the beads being able to select the phage of interest an only captures progeny phage produced from the first round of binding to the bead that produced in the host cell (col. 25, lines 37-50). As in claim 14, Janda teaches going from room temperature during infection to 37 degrees C during incubation (col. 25, lines 37-50).

5. I disagree with the Office's position. The element of producing or amplifying phage in less than 4 hours would not have been obvious in light of the '681 patent, the '484 patent, or the '419 patent (alone or in combination).

*Janda*. The passage the Office points to in the '681 patent discloses:

> Phage are removed and each well is then washed with TBS/Tween (50 mM Tris-HCl at pH 7.5, 150 mM NaCl, 0.5% Tween 20) for five minutes at room temperature. The plate is washed with distilled water, and noncovalently adherent phage are eluted by the addition of 50 ul of elution buffer (0.1M HCl, adjusted to pH 2.2 with solid glycine) to each well and incubation at room temperature for five minutes. Covalently bound phage are eluted in two separate 50 μl washes with 20 mM DTT at 25C. The resulting DTT-eluates from each of the six libraries are combined.
>
> Eluted phage are used to infect 2 ml of fresh ($OD_{600}$ =1) E. coli XL1-Blue cells for 15 minutes at room temperature, after which 10 ml of SB containing 20 ug/ml carbenicillin and 10 ug/ml tetracycline is admixed. Aliquots of (20, 10, and 1/10 ul are removed for plating to determine the number of phage (packaged phagemids) that are eluted from the plate. The culture is shaken for 1 hour at 37C, after which it is added to 100 ml of SB containing 50 ug/ml carbenicillin and 10 ug/ml tetracycline and shaken for 1 hour. Helper phage VCSM13 ($10^{12}$ pfu) are then added and the culture is shaken for an additional 2 hours. After this time, 70 ug/ml kanamycin is added and **the culture is incubated at 37C overnight.** Phage preparation and further panning/affinity selections are repeated as described above. (col. 25, lines 27-50; emphasis added)

As this passage makes clear, the phage-related methods of the '681 patent require that phage-infected XL1 cells be grown overnight. Further, even prior to the overnight incubation step, the cells are incubated for additional periods of time: the phage-infected XL1 cells are incubated for an hour, then the phage-infected XL1 cells are added to 100 ml of SB and incubated for another hour, then helper phage are added to the culture containing the phage-infected XL1 cells, and the helper-phage infected culture is incubated for two hours. Thus, in light of the '681 patent's disclosure, a skilled practitioner would incubate phage-infected cultures for longer than overnight.

*Ladner*. Next, the '484 patent also teaches incubation times greater than 4 hours. The relevant passage in the '484 patent is at col. 144, lines 17-35. As stated therein:

> We added 1.1 . $10^8$ plaque forming units of the KLMUT library to 10 µl of a 50% slurry of agarose-immobilized human neutrophil elastase beads (HNE from Calbiochem cross-linked to Reacti-Gel™ agarose beads from Pierce Chemical Co. following manufacturers directions) in TBS/BSA. Following 3 hours incubation at room temperature, the beads were washed and phage was eluted as done in the selection of EpiNE phage isolates (Example IV). The progression in lowering pH during the elution was: pH 7.0, 6.0, 5.0, .4.5, 4.0, 3.5, 3.0, 2.5, and 2.0. **Beads carrying phage remaining after pH 2.0 elution were used to infect XL1-Blue™ cells that were plated to allow plaque formation.** The 348 resulting plaques were pooled to form a phage population for further affinity selection. A population of phage particles containing 6.0 . $10^8$ plaque forming units was added to 10 µl of a 50% slurry of agarose-immobilized HNE beads in TBS/BSA and the above selection procedure was repeated. (emphasis added)

The '484 patent teaches that phage-infected XL1-Blue™ cells are plated to allow plaque formation. For the type of phage used in these experiments, in the plated XL1-Blue™ cells, plaque formation takes longer than 4 hours to occur. Indeed, even under favorable growing temperatures (e.g., 37°C), plaque formation typically occurs after **about 12-18 hours**. Thus, in view of the '484 patent, a skilled practitioner would understand that phage-infected cells would need to be incubated for longer than 4 hours.

*Anderson*. The disclosure in the '419 patent is limited with regard to methods involving phage. For example, as stated at col. 23, line 65 to col. 24, line 8:

> The protein-coated beads are also used for determining and/or purifying a receptor from a combinatorial library of compounds or receptor display microorganisms or cells. As such, the beads provide a solid phase substrate for separation. Of particular concern are antibody display phage libraries constructed from immunoglobulin sequences obtained from naive animals or from animals that have been immunized to the protein or a mixture of proteins. The phage which bind to the beads may be later eluted and cultured to produce large quantities of protein binding receptor.

Anderson fails to describe or suggest any time period for incubating phage-infected cells.

Thus, in my opinion, in light of this combination of references, it would not have been obvious to perform the step of producing or amplifying phage in less than 4 hours, as recited in the claims. The references teach that much longer incubation times are needed.

6. In my opinion, the advantages provided by the methods recited in the amended claims are significant. For example, as described in the application, using methods known in the art, *a single round* of selection takes *one to five days* to complete (see, e.g., par. bridging pages 1-2). As a result, if multiple rounds of selection are performed, the process would take well over a week. In large part, this is because such methods require that after selection against a target, phage that bind to the target are used to infect host cells and the phage-infected cells are grown for long periods of time, typically overnight. After the overnight growth, the phage must be pooled and/or purified before being used for another round of selection. Pooling and/or purifying steps further lengthen the total time needed to complete the selection procedure.

In contrast, the claimed methods allow for <u>multiple rounds</u> of selection to be completed in a <u>single day</u> (see, e.g., page 15, first full par.). For example, as described in the application:

> One of the advantages of some implementations described here is a greatly reduced time for performing multiple cycles of binding selection. Unlike some methods, the number of input phage in each successive cycle is reduced. For example, subsequent cycles can use a number of input phage that is at least one, two, or three orders of magnitude less than the initial cycle or the previous cycle. **The amplification of binding phage in the presence of the target compound is one method for saving time.** (page 14, fourth par.; emphasis added)

An example of the methods of the application is described as follows:

> In one embodiment, the method includes amplifying a display library member in the presence of a target compound. The method can be used to identify members of the library that interact with the target compound. **In another embodiment, the method restricts amplification to a limited interval or a burst so that the selection process rapidly advances, e.g., through successive cycles**. Still other features are disclosed that enable improved selection of the display library. As noted above, the methods are applicable at least to bacteriophage and to phagemids, as well as other types of display libraries.
>
> One exemplary method includes the following:

> a. Contacting a plurality of diverse display phage to a target compound;
> b. Separating phage that bind to the target compound from unbound phage;
> c. Infecting host cells with the bound phage;
> d. Producing replicate phage from the infected cells in the presence of the target compound ("phage production");
> e. Separating phage that bind the target compound from the unbound phage and infected cells;
> f. Repeating c. to e. one or more times, e.g., one to six times;
> g. Recovering the bound phage, e.g., for individual characterization.
>
> **In the above example, each round of replicate phage production is carried out in the presence of target compound.** The host cells are typically $F^+$ *E. coli*. The *E. coli* are maintained in the presence of the target compound during infection and during phage production by the infected cells. **The cells can be maintained, e.g., for less than three division cycles, e.g., less than an hour. As soon as replicate phage emerge from the infected cells, they can bind to the target compound since the target compound is present during phage production. Moreover, it is not essential to purify or harvest the emerging phage during each cycle.** (page 16, second-fourth pars.; emphasis added)

The working examples of the application show the use of such methods. For example, in Example 1 (beginning at page 36), phage-infected bacteria were incubated for 45 minutes (e.g., less than 4 hours).

7. Summary: In my opinion, the significant advantages afforded by the claimed methods could not have been arrived at by routine experimentation, but instead required inventive thought. As the above-quoted passages from the '681 patent, the '484 patent, and the '419 patent make clear, traditional methods of selection require laborious and time-consuming steps that are minimized or avoided by the present methods.

For example, in the '681 patent, phage that bound to target were eluted. Then the eluted phage were used to infect bacterial cells, and the cultures were incubated for longer than overnight. The next day, the phage had to be prepared from the overnight cultures and then another round of selection with fresh target could be initiated.

In the '484 patent, phage that bound to target were used to infect bacterial cells, the phage-infected bacterial cells were incubated until plaques formed. The plaques had to be pooled, and then another round of selection with fresh target could be initiated.

The '419 patent provides more limited teachings, fails to describe or suggest any time for incubating phage-infected cells, and indicates that any phage that bind to target have to be eluted. The '419 patent merely discloses that the phage must then be cultured to produce "large quantities."

In contrast, the claimed methods minimize or avoid such steps. For example:

- the claimed methods do not require long (e.g., overnight) periods of incubation after bacteria are infected with target-specific phage;

- the claimed methods do not require preparation or pooling of phage from the bacterial cells;

- the methods do not require large quantities of phage to be produced.

Indeed, Applicants have discovered, *inter alia*, that target-specific phage can be used to infect bacteria, even when in the presence of target. No elution step is required. Further, no phage preparation or pooling step is needed after phage-infected bacteria are cultured. Of particular significance, Applicants have discovered that large quantities of phage from a given round of selection do not need to be prepared prior to initiating a subsequent round of selection. As a result, dramatically reduced incubation times can be employed after bacteria are infected with phage. Indeed, not only can the cultures be incubated for less than 4 hours, but even times of less than 2 hours or 1 hour can be sufficient. In my opinion, methods with these advantages could not have been easily arrived at using routine experimentation.

8. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Date: 2009.09.16

Robert Charles Ladner

Robert Charles Ladner, Ph.D.